How omics technologies can enhance chemical safety regulation

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How OMICs Technologies Can Enhance Chemical Safety Regulation: Academia, Government and Industry Perspectives

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Abstract:	The current rapid pace of innovation of industry is delivering an ever-increasing number and diversity of chemicals within industrial and consumer products. Regulations require an evaluation of the environmental and human hazards of chemicals, ultimately characterizing the risks associated with their manufacture, use and disposal. In order for policy makers and regulators to carefully balance economic priorities with the need to protect vital ecosystems and public health, equally novel, revolutionary and coordinated scientific approaches to hazard and risk assessment are needed. Such approaches not only need to be robust and reliable they also need to be time and cost effective whilst avoiding, wherever possible, the use and reliance on animal testing. There are times in every field of science when technological advances set the stage for progress at a pace that was previously inconceivable. For regulatory science, the time is now. The goal of this Perspectives column is to discuss how stakeholders foresee the use of OMICs data to trigger a genuine and fundamental change to redress the escalating challenges faced by industries, governments and the public in the assessment of potential health and environmental hazards imposed by thousands of un(der)tested

	chemicals. We ask: How the different stakeholders see the current use of OMICs for chemical safety assessment and what are the critical advances required so it can deliver valuable solutions to improve our confidence in chemical safety assessment and ultimately be incorporated into global regulatory frameworks?

How Omics Technologies Can Enhance Chemical Safety Regulation: Academia, Government and Industry Perspectives

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Acronyms

RA	Risk assessment
MoA	Mode of Action
WoE	Weight of Evidence
NC3Rs	National Centre for the Replacement Refinement & Reduction of Animals in Research
QSAR	Quantitative structure–activity relationship
IATA	Integrated Approach to Testing and Assessment
TK/TD	Toxicokinetic-Toxicodynamic
CBR	Critical Body Residue
PEC	Predicted Exposure Concentration
GC-MS	Gas chromatography–mass spectrometry. An analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample.
RNA-seq	Ribonucleic acid sequencing. A transcriptome sequencing technique used to reveal the presence and quantity of RNA molecules in a biological sample at a given moment in time.

Lead

Bruno Campos and John k. Colbourne

The current rapid pace of innovation of industry is delivering an ever-increasing number and diversity of chemicals within industrial and consumer products. Regulations require an evaluation of the environmental and human hazards of chemicals, ultimately characterizing the risks associated with their manufacture, use and disposal. In order for policy makers and regulators to carefully balance economic priorities with the need to protect vital ecosystems and public health, equally novel, revolutionary and coordinated scientific approaches to hazard and risk assessment are needed. Such approaches not only need to be robust and reliable they also need to be time and cost effective whilst avoiding, wherever possible, the use and reliance on animal testing. There are times in every field of science when technological advances set the stage for progress at a pace that was previously inconceivable. For regulatory science, the time is now. The goal of this Perspectives column is to discuss how stakeholders foresee the use of OMICs data to trigger a genuine and fundamental change to redress the escalating challenges faced by industries, governments and the public in the assessment of potential health and environmental hazards imposed by thousands of un(der)tested chemicals.

We ask: How the different stakeholders see the current use of OMICs for chemical safety assessment and what are the critical advances required so it can deliver valuable solutions to improve our confidence in chemical safety assessment and ultimately be incorporated into global regulatory frameworks?

an Academia perspective

James B. Brown and Mark R. Viant

State of the science

The current level of technological maturity attained by diverse platforms like Next Generation Sequencing as well recent advances in chromatography and mass spectrometry have enabled the high–content and high-quality molecular interrogation of biological systems. The process of comprehensively measuring, or, deeply sampling a molecular landscape is known as “Omics”. Dozens of “omes” are now open to study: the epigenome (heritable modifications to DNA or chromatin that do not alter the underlying genetic sequence); the transcriptome (RNA); the proteome (proteins); the metabolome (usually defined as all small molecules present in a system). It is now possible to globally survey gene products with single-cell resolution, to simultaneously measure hundreds of proteins and thousands of metabolites, and to use time series measurements to infer the relationships between these processes, as well as with tissue and organismal phenotypes [1, 2].

‘OMICs’ have fundamentally transformed the approach used by basic scientists for investigating and characterising molecular processes in plants, animals and microbes [3]. Comprehensive information about biological systems has effectively industrialized the process of hypothesis generation: data mining procedures have leveraged advances in statistical machine learning to discover new molecular processes and pathways from the integrative analysis of multi-OMICs surveys [4]. High-profile data-driven discoveries based on the analysis of OMICs data include the role of epigenetics in transmitting the maternal “memory” of transcription to the egg and the molecular basis of over two thousand distinct genetic diseases. In principle, OMICs are therefore ideal tools for discovering key events (KEs), within Adverse Outcome Pathways (AOPs) [5]. If sufficiently reproducible, robust, and harmonized protocols can be developed for the characterization of chemical exposures in relevant and ethically credible biological and ecological systems, OMICs approaches are

likely to emerge as the most plausible solution to the international chemical safety crisis.

Public demand for safer environments has been growing, and sophisticated regulatory frameworks governing the manufacture and dispersal of chemicals are now in place throughout the world. In Europe, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH, EC 1907/2006) requires safety assessment and risk management for all chemicals used in quantities of more than one tonne per year, and the Water Framework Directive (WFD, 2000/60/EC) requires assessment and management of ecological and chemical water-quality for inland, transitional and coastal water bodies in Europe. In the United States, The Frank R. Lautenberg Chemical Safety for the 21st Century Act (H.R.2576, 114th Congress 2015-2016) has explicitly modernized toxic substances testing requirements by opening the door to Integrated Approaches to Testing and Assessment (IATA) in regulatory science. To support these innovative legislations, novel and coordinated assessment procedures that are rapid, cost effective, and consistent with ethical standards are urgently needed. A proposed solution is the application of data-rich OMICs technologies in non-mammalian organisms coupled with *in vitro* human-derived models to systematically identify the MoA's of compounds. A collection of pioneering studies have demonstrated the contribution of OMICs in a weight-of-evidence approach helping the formulation of AOPs [6], and now these and related studies need to be replicated to establish robust, transferable, and reliable protocols for chemical safety assessment.

Proposed roles for OMICs technologies in chemical safety assessment

Accepting for a moment the premise that 'OMICs technologies can, in the near term, contribute significantly to the discovery of the modes of action of chemicals; we foresee three specific regulatory applications for this new knowledge. **First**, KEs discovered from 'OMICs measurements and then biologically validated in subsequent targeted studies could enable biologically

based read-across – leading to more reliable and cost-effective prioritization of chemicals for testing, as well as more accurate hazard prediction [7]. To achieve this, chemicals would first be grouped according to the molecular KEs that they activate, then the apical toxicity of some chemicals within a given chemical group could be predicted from the known adverse outcomes of the more extensively studied chemicals in that group. Read-across (of apical toxicity) based upon the activation of a panel of molecular KEs exploits far richer information than is presently used for quantitative structure-activity relationship (QSAR) analysis. **Second**, molecularly defined AOPs could lead to improved cross-species extrapolation. In this emerging field, we seek to apply the principles of evolutionary biology to toxicology – to identify conserved (or not) biochemical pathways that give rise to susceptibility or resiliency to compounds. This is an essential step, because it could enable the translation of tests conducted in tractable model organisms to the entire tree of life. **Third**, and most simply, given the capacity of OMICs-enabled studies to identify KEs, we expect rapid growth in the number of OECD-approved AOPs – which is already apparent: at the time of writing, there were only six OECD endorsed AOPs, and 113 under development. The stage is set for an exponential growth in the collection of adverse outcome pathways with extensive molecular characterisation in human and other metazoan systems.

However, a key question remains: how predictive are the OMICs-based test data of higher levels of biological organisation (the adverse outcome), including growth and reproduction of organisms and population or ecosystem fitness? It is increasingly clear that OMICs will radically enhance our capacity to discover molecular predictors of adverse outcomes at the level of individuals [8], but there is still little evidence to suggest that we can extrapolate to populations or ecosystems. Transgenerational studies are beginning to show promise [9], but ecological toxicology lacks the basic tool that serves as a foundation to virtually all other biological science: the model system. We have model organisms, model *in vitro* tissue cultures, but no model ecosystems. Mesocosms are a step in the right direction, but are generally too expensive and slow to function on time scales relevant to industry or government. Ecosystem models (in silico

and/or in vivo) for toxicity testing as reproducible and rapid as the development of the model organism *Drosophila melanogaster* or *Daphnia spp.* are needed to enable this leap. Will these be freshwater microcosms? 3D-printed terrestrial systems? A number of *consortia* are now racing to build such constructs (http://eco-fab.org/) and we will be watching their progress with interest.

How to progress OMICs based safety assessments operationally

While many academics remain convinced of the power of 'OMICs technologies in regulatory toxicology, several challenges do remain. For example, while 'OMICs have a proven record of discovering novel molecular mechanisms of disease, aging, and toxicity, a standardized design of the OMICS study (particularly the design of the method performance criteria) and the statistical/computational strategies for discovering molecular KEs have not yet been established. The distillation of OMICs approaches to precisely defined and exquisitely reproducible protocols has yet to happen, and while some cross platform validation has already taken place [10], much more is needed. Bioinformatics and statistical tools also need to be standardized, and entire pipelines, from the lab bench to quantitative conclusions, need to be packaged and made distributable. But this is no easy task – credible use of these technologies will require massive efforts towards validation and standardization. In the genomics community, the Encyclopaedia of DNA Elements Consortium (ENCODE), a multinational, NIH-funded endeavour that has cost over a quarter of a billion dollars, operated since 2003, and has been a major contributor to the standardization of analytical pipelines in the genomics community (https://www.encodeproject.org/). We need to scale up our commonly artisanal projects to validate findings and procedures in "ring tests" ensuring and quantifying reproducibility. At a minimum, a collection of case studies need to be conducted at four or more laboratories with high biological replication, from data generation to knowledge extraction. We posit that such case studies, along with effective cross-disciplinary and cross-sector communication and education, are the necessary foundation of future regulation and policy. More broadly, the community needs to think collaboratively – and grandly – to imagine and

establish ENCODE-scale Consortia suited to tackling the enormous challenges presented by the science of molecular toxicology.

Although substantial challenges remain, we suggest that a clear goal has come into view for the future of OMICs in regulatory science: the generation of a publicly owned Knowledge Base organizing and distributing data and conclusions derived from molecular and OMICs studies. Here we don't refer to (existing) repositories of experimental OMICS data, such as GEO or MetaboLights, rather a database of molecular measurements derived from standardized assays of highly informative KEs organized in a semantic way enabling chemical producers to query molecular responses in model systems for new compounds against a library of existing test data, and make projections for the activity of the new compound across the entire tree of life – a tantalizing prospect. Such a resource stands to provide enormous dividends for both public and private sectors.

Given the immediate legislative needs for setting regulatory priorities and the economic benefits of manufacturing safer products by design, now is the time to apply high-throughput multi-OMICs technologies for 3R-compliant toxicity assays. The SETAC community can and should be key part of this effort, and should further coordinate global efforts to achieve the objective of migrating towards 21st century solutions to (eco)toxicology.

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A Government Perspective

Adam D. Biales, Kathryn Gallagher, Tala R. Henry, Keith G. Sappington

State of the science

Despite decades of toxicological research and advances in the chemical regulatory landscape throughout the world, the need for improving the efficiency and accuracy of our chemical risk assessment process has never been greater. Characterizing chemical risk is accomplished through the linkage of measured or modeled exposure and toxicity values. The current paradigm for risk estimation has limitations based on the types of tools available, as well as the availability of data to expand the existing tools and/or create new, improved tools. Traditional methods are ill equipped to robustly assess risks associated with the 140,000+ chemicals in commerce worldwide in an efficient and timely manner, let alone other perennial issues such as mixtures. It has been suggested that the various OMICs modalities, such as transcriptomics, proteomics, metabolomics and epigenomics, may be used to augment traditional methods, or be combined with other next generation technologies to address limitations and provide a better characterization of chemical risk [1, 2]. Alterations on the molecular, biochemical, and/or cellular levels of organization, typically measured using OMICs technologies often represent the initial responses of organisms to a chemical exposure and are thought to be initiating events in the pathway to adverse changes on higher biological levels. Many of the technological platforms available (e.g., microarrays, GC-MS, RNA-seq) are able to measure changes across large proportions of the total OMICs response of an organism, tissue, or cell (i.e., 100s to 1000s of genes, proteins, or metabolites). The sheer number of simultaneously measured endpoints can be applied to chemical risk assessment in any number of ways (discussed below). Lastly, OMICs-based approaches are amenable to high and medium throughput experimental formats, suggesting their utility for the rapid screening and characterization of untested chemicals.

Potential of OMICs technologies in environmental regulation

The high dimensionality of OMICs data sets suggests the possibility of developing OMICs-based fingerprints for a chemical or activated biological pathway. These fingerprints have the potential to be applied to both exposure and hazard assessment in an unsupervised or non-targeted manner to simultaneously screen all activated biological pathways within in vivo or in vitro systems, requiring no a priori information regarding mode of action (MOA) [3, 4]. Though there are few, if any examples, of these tools being used in a strict regulatory framework, there are several examples demonstrating their potential within the human clinical world where they have been shown to outperform gold standard tests and have reached the lofty bar of FDA approval [5, 6]. The ability to develop MOA-specific fingerprints makes this concept amenable to discovery and characterization of multiple cellular pathways simultaneously in a single experiment, as opposed to screening against a panel of focused bioassays, providing needed time and resource efficiency. Throughput, however, is highly dependent on the development of automated pipelines for data analyses and interpretation. The ability to screen for all activated MOA simultaneously has particular benefits in characterizing real-world exposures. Environmental samples are often highly complex mixtures, with chemical constituents that have the potential to interact, altering both toxicity and exposure parameters. Mixture constituent interactions are not reflected in analytical chemistry measures; however, the OMICs endpoints are effects-based measures, thus they should effectively integrate potentially confounding factors. Fingerprints can also be used for hazard assessment, where they can be used to identify MOA of untested or uncharacterized chemicals by establishing relationships among chemicals based on similarities of their OMICs responses [7]. OMICs-based fingerprints can also be related to the manifestation of disease states, suggesting the potential for predictive measures of apical response. An extension of this is the ability to leverage publicly available datasets, reducing the need for additional toxicity testing. The utility of these existing datasets may be further increased by the possibility to extrapolate across experimental platforms, cell lines and species, suggesting the potential for rapidly increasing the coverage of the total chemical space without the need for further testing [8].

Regulatory challenges potentially addressed using OMICs technologies

The relatively high costs and lengthy timeframes associated with producing measured toxicity data puts practical limits on the number of conditions that can be reasonably tested. This subsequently introduces uncertainties when extrapolating beyond the relatively simplistic laboratory conditions. Measured toxicity data exists for only a limited number of chemicals relative to the total number of chemicals in commerce, and generally focus on the discrete parent compounds or commercial mixtures as manufactured rather than their metabolites or environmental degradation products and employ a limited set of exposure conditions. Because OMICs experimental platforms are amenable to high throughput formats (think TOXCAST®, NGS, among others), it is possible to rapidly and efficiently expand testing to cover a greater range of experimental conditions, chemical formulations or mixtures, and metabolites which should lead to reduced uncertainty in assessing chemical hazard and risk. Additionally, OMICs endpoints may be most informative when taken relatively soon after the exposure is initiated, as these responses are more readily associated with the particular chemical exposure rather than somewhat generic pathways leading to apical outcome(s). Comparatively rapid OMIC-based assays further contribute to the potential for a highly efficient and relatively inexpensive platform that would allow a greater coverage of the total chemical space and allow testing to expand beyond the parent compound and include an increased set of exposure conditions. OMICS measures taken in exposed cells, organs or organisms should capture all biological pathways that are altered by a chemical or environmental sample. Their ability to integrate across MOA suggests that they may have increased utility relative to traditional bioassays targeting single events, such as receptor binding, single gene or enzymatic activation. Many currently used bioassays focus on initial single events, such as direct binding of a ligand to its cognate receptor. As a result, they may not be able to identify indirect effects or activation of a particular MOA at a point downstream of receptor binding. Finally, as many components of biological pathways are well characterized, changes in expression of many components of a biological

pathway may provide an increased weight of evidence suggesting a particular MOA.

The same practical realities that limit our ability to explore more varied exposure scenarios, also limits the number and variety of species that we can test. Regulatory programs generally intend to be protective of the majority of species likely to be exposed. Given the huge diversity of species and the fact that toxicity data are lacking for most species, it can be difficult to quantify the degree of uncertainty underlying the assumption. Similarly, toxicity tests conducted in one life stage may not capture vulnerabilities of other life stages, as exposure routes and sensitivities may differ [9, 10]. OMICs may provide a means to extrapolate from model species to less characterized species. Though chemical-induced OMICs profiles may be related to MOA, they may also provide insights into compensatory mechanisms (clearance, metabolism, etc). Susceptibility, at least in part, may therefore be "coded for" in the sequences of genes related to xenobiotic metabolism and clearance or directly to the MOA [11-13]. The cost of sequencing genomes continues to decrease, which is resulting in a huge increase in the number of non-model species with sequence level information [14]. It may be possible to leverage sequence level data and epigenetic modifications in combination with life history information and phylogenetic relationships to predict sensitivities of untested organisms. Sequence data has been used to accurately predict sensitivities across species [15] as has phylogeny [16].

Challenges and limitations of OMICs technologies in environmental regulation

Though OMICs show great promise in a myriad of applications related to chemical risk, there are limitations that must be addressed before their use in regulatory settings. The majority of OMICs-based studies are aimed at discovery rather than at the development of tools useable within regulatory contexts. The performance of OMICs-based tools must be characterized and their applications standardized under the conditions that they will likely be employed. For the application of OMICs data and OMICs-based tools to emerge/evolve, it is critical for these data/tools to be put into practical and "fit-for-purpose" assessment framework(s) and that they demonstrate an added

benefit to the currently used measurement/assessment endpoints. Another potential limitation of OMICs-based tools is the difficulty in interpreting OMICs change relative to risk, as they are often reversible and are early events in the pathways leading to higher order adverse outcomes. For OMICs-based data/tools to be effectively used to predict adverse outcomes, it will be critical to establish quantitative relationships among the events along the adverse outcome pathway and with the apical endpoint of regulatory concern. These tools must be honed to their intended regulatory application; however, it must be recognized that OMICs-based tools may not easily lend themselves to traditional means of estimating chemical risk and for them to be maximally useful we must consider adapting practices to accommodate them. For example, many regulatory actions rely upon quantitative concentrations as a trigger for action or for protective values, yet it is not clear if OMICs endpoints are amenable to a single value. Rather they may provide more semi-quantitative data (high, medium or low) or even qualitative data, which, in combination with other toxicity measures may be result in a greater weight of evidence supporting the likelihood of an adverse outcome. How these can be incorporated into environmental regulation will require close communication between the regulatory community and those developing the OMICs-based tools and is somewhat constrained by the statutes that the regulatory community is tasked with implementing.

Disclaimer: The views expressed in this article are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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An Industry Perspective

Stuart Marshall and Graham Whale

State of the science

Industry has responsibility to assure safe production, handling, use and disposal of chemicals. It does this through assessing the associated hazards and risks and by implementing relevant risk reduction measures. Companies have an ethical responsibility for this irrespective of regulatory requirements. Therefore, industry is keen to see development of science-based assessment of chemicals for both internal safety assessments and in regulatory frameworks. Hazard assessment in current regulatory frameworks was founded on testing representative or surrogate organisms in vivo. A key incentive for the development of sub-individual level assessment methods such as OMICs is the drive towards animal alternative methods, i.e. assessing hazardous properties without using protected stages of vertebrates. Depending on the level of testing and refinement needed, in vivo testing can be cost and time intensive. Alternative methods are desirable but it is important that there is confidence and reassurance that assessments are fit for purpose and that approaches can be easily communicated to relevant stakeholders.

The question of using OMICs in Risk Assessment (RA) is not new [1, 2] but developments over recent years have accelerated progress, especially in the capability for using gene expression data that cover a large proportion of cellular components to identify potential biomarkers and in advanced bioinformatics and statistical methods to discriminate control versus treated molecular responses. The availability of a limited number of fully sequenced organisms (including fish, daphnia, nematodes, slime moulds and algae), the high throughput nature of the technology and data processing capacity now bring opportunities for the rapid assessment of chemicals using several types of OMICs assays. Guidance on data collection and processing is also developing to improve the quality and robustness of OMICs screening tests (MERIT (MEtabolomics standaRds Initiative in Toxicology) www.ecetoc.org). Commercialisation of the technology is helping to reduce variability. However,

even with these advances, many of the approaches are still being used as research rather than RA tools. This is due to a range of factors such as lack of standardisation and complexities in interpreting data in terms of representing apical endpoints. Nevertheless, there is a need for lower cost, more rapid, less animal intensive studies to help screen potential new products in order to identify those which raise concerns and may require additional assessment. Such approaches are also required to help underpin chemical grouping or category approaches for UVCB substances. The Cat-App project, initiated and funded by CONCAWE, is investigating practical strategies for grouping and read across approaches by developing a framework based on chemical-biological read-across [3]. The approach is to integrate innovations in (i) in vitro testing, (ii) high-throughput genomics and (iii) integrative data analyses and visualisation into a transparent workflow for read-across assessment of UVCBs in regulatory programmes (https://www.concawe.eu/cat-app/).

Opportunities for OMICS technologies in chemical hazard and risk assessment

OMICs technology is unlikely to address the challenges of increasing the environmental relevance of RA, i.e. assessing protection goals set at population, community and ecosystem levels [4]. Indeed, even at the individual level, more knowledge of systems biology is required to link molecular responses to physiological processes at increasing levels of complexity, covering all life stages. This includes the knowledge needed to interpret OMICs data in terms of adaptive or adverse effects e.g. quantitatively linking up/down regulation to apical endpoints via plausible biological pathways and accounting for temporal changes in signals. Other influences on OMICs responses such as environmental factors (water quality, temperature, food) and the genetic variability of a species - both inter-individual variation in expression and differences between lab and field populations, also require consideration although it should be noted that this is not made in most conventional testing guidelines. These confounding influences mean that OMICs assays must be well standardised and validated in terms of replicability, reproducibility and repeatability. Consequently, in the near to mid-term, expectations for applying OMICs in RA may be tending away from the prediction of apical endpoints

towards their use in identifying and understanding KE as part of an integrated approach to testing and assessment (IATA).

Overcoming the considerable challenges in applying OMICs to RA will only occur if there is confidence in the techniques and clarity on the applicability domains. Increasing confidence in RA may be achieved through a WoE approach to hazard identification and characterisation e.g. using existing ecotoxicity data, QSARs, chemical grouping (by structure and bioassay responses), TK/TD modelling and generating new in vitro or in vivo ecotoxicity data. OMICs data are starting to play a useful role in by providing supporting information to help explain differences in species sensitivity, for use in qualitative grouping/read-across and in the determination of MoA.

Determination of MoA is an important and useful input to a RA strategy. It would be beneficial to develop a library of MoA fingerprints for a range of species to compare with OMICs response data to test chemical exposure. These fingerprints would need to be resolved and described at a suitable level, e.g. for a range of tissues, sexes, life stages and account for changes in response over time. If response patterns are sufficiently similar, then it may be possible to infer the same MoA. For example, if a chemical could be categorised as a baseline toxicant then knowledge of the internal critical body residue (CBR), for this generic MoA could be used to simplify the RA by comparing the CBR with external exposure (PEC) using conservative TK models or chemical activity [5]. However, OMICs may be more effective in assessing chemicals with a specific MoA/receptor where a characteristic OMICs response can be identified. This information could add to conventional structural approaches, e.g. to assessing chemicals with specific Moa's [6]. As previously discussed, these data could also be used to categorise chemicals; the value of OMICs for this purpose is currently being investigated by CONCAWE to assess and confirm petroleum product categories under the EU REACH regulations.

Using OMICs to reduce animal use in health and environmental risk assessments

OMICs data are also showing promise in understanding species differences in sensitivity to chemicals with specific Moa's, e.g. by searching for target genes across species (gene homology) [7]. Identifying conserved key genetic pathways has promise in enabling non-vertebrates such as nematodes to be used to provide hazard screening data for fish and mammals.

In terms of models, fish are vertebrates and therefore share a high degree of sequence and functional homology with mammals, including humans. Due to the conservation of cell biological and developmental processes across all vertebrates, studies in fish can give great insight into potential effects on higher vertebrates and even human disease processes. For example, studies with proteins have shown these have a similar function in fish and mammals [8]. In addition to fish other alternative developmental biology model systems are currently being explored, ranging from whole organism systems with nematodes and slime moulds, to cell based systems using mouse or human embryonic stem cells. Groups working with these systems are embracing and exploiting recent developments in molecular biology and genetics to better understand the underlying pathways driving normal embryogenesis and the effects of perturbations to these pathways. For example, the potential of some of these test systems has recently been investigated as part of an NC3Rs challenge (www.crackit.org.uk/challenge-10-predart). Using research from this challenge from nematode (Caenorhabditis elegans) and zebrafish models have been shown to have potential to screen for mammalian relevant pre-DART (development and reproductive toxicity) phenotypic endpoints [9]. The pre-DART challenge omics results also indicated DART specific responses on RNA and protein level and that 4- RNAi in C. elegans indicated critical genes for toxicological response although these results are yet to be published. However, building on aspects of this research the UK NC3Rs launched a new DART paths challenge which aims to develop a cross-organism mapping strategy and framework (https://crackit.org.uk/challenge-26-dartpaths). Industrial companies with policies in place to reduce animal use are supporting these initiatives to help unlock the potential of these alternative test systems. It is envisaged that this will be achieved by improving the understanding of conservation of key pathways across different species and identifying and linking genes with the

same function and role in driving DART between organisms. Although the current focus is on health endpoints these test systems have relevance for ecological endpoints as they can inform on potential concerns related to longer term effects of chemicals in fish as well as potential concerns related to mammals. In the future, such test systems could potentially provide more holistic health and environmental assessments of environmental samples (e.g. in contaminated site, surface water quality and plant protection product field investigations).

In summary, there are a number of exciting opportunities for the application of OMICs technologies within health and environmental risk assessments. They offer potential to provide high throughput more holistic screening assessments with reduced reliance on traditional in-vivo (i.e. using 'protected stages) vertebrate models. However, the challenges to application of OMICs in such assessments are broad and numerous. Overcoming these challenges will require a co-ordinated effort across academic, industry and regulatory sectors with the specific aim of applying/implementing the technology in chemical safety and environmental risk assessments.

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